REMARKS/ARGUMENTS

Claims 28-32 are pending in this application.

I. Claim Rejections Under 35 U.S.C. §101 and 35 U.S.C. §112, First Paragraph

Claims 28-32 remain rejected under 35 U.S.C. §101 because the claimed invention allegedly "is not supported by either a specific and substantial asserted utility or a well established utility." (Page 2 of the instant Office Action).

Claims 28-32 are also rejected under 35 U.S.C. §112, first paragraph, allegedly "since the claimed invention is not supported by either a specific and substantial asserted utility or a well established utility for the reasons set forth above, one of skill in the art clearly would not know how to use the claimed invention." (Page 2 of the instant Office Action).

For the reasons outlined below, Applicants respectfully disagree and traverse the rejection. With respect to Claims 28-32, Applicants submit that not only has the Patent Office not established a *prima facie* case for lack of utility and enablement, but that the PRO1303 polypeptide and the claimed antibodies that bind it possess a credible, specific and substantial asserted utility, and based on this utility, one of ordinary skill in the art would know how to use the claimed antibodies without further experimentation.

The results of the adipocyte glucose/FFA uptake assay provide utility for the PRO1303 polypeptide and the claimed antibodies that bind it

Applicants respectfully submit that they rely in part on the adipocyte glucose/FFA uptake assay) for patentable utility of the PRO1303 polypeptide and the claimed antibodies that bind it, and that the adipocyte glucose/FFA uptake assay data for the PRO1303 polypeptide is clearly disclosed in the instant specification under Example 149.

The adipocyte glucose/FFA uptake assay is designed to determine whether a polypeptide is capable of modulating the uptake of glucose or free fatty acids in adipocyte cells. By making such determinations, the assay identifies polypeptides that are expected to be useful for treating disorders wherein stimulation of glucose uptake by adipocytes is expected to be therapeutically effective. Examples of these types of disorders include obesity, diabetes and hyperinsulinemia.

PRO1303 resulted in more than 1.5 times the uptake of the insulin control, and therefore PRO1303 tested positive as a stimulator of glucose/FFA uptake in adipocyte cells.

As discussed in Applicants' Appeal Brief, the glucose/FFA uptake assay as described in Example 149 of the instant application was also well known in the art at the time of the effective filing date of the instant application. Similar assays were commonly used to identify potential anti-diabetic agents and to study the regulatory mechanisms of important molecules involved in fat cell metabolism. For example, at the time of the effective filing date of the instant application, it was well known in the art that increasing glucose uptake by adipocyte cells is a hallmark of a number of therapeutically effective agents, such as troglitazone and pioglitazone. (Tafuri; Sandouk, *et al.*, made of record July 5, 2005). Using a rat adipocyte culture system similar to the system disclosed in the instant application, Goldwaser *et al.* (made of record July 5, 2005) showed that vanadium ligand l-Glu (γ)HXM potentiates the capacity of free vanadium ions to activate glucose uptake and glucose metabolism in rat adipocytes *in vitro* by 4-5 fold and to lower blood glucose levels in hyperglycemic rats *in vivo* by 5-7 fold. In addition, the investigators in Mueller *et al.* (made of record July 5, 2005), who were interested in determining the influence of glucose uptake on leptin secretion, employed essentially the same assay to measure changes in glucose uptake after insulin exposure. Using the same assay system, Mueller *et al.* further studied the effect on leptin secretion of two well-known anti-diabetic agents, metformin and vanadium, which were known to enhance glucose uptake.

Accordingly, Applicants respectfully submit that at the effective filing date of the instant application, one of skill in the art would have reasonably accepted that various compounds, such as PRO1303, that are capable of modulating glucose uptake have a substantial, practical, real life utility. The above-mentioned studies have clearly established that the glucose/FFA uptake assay as described in the instant application is a reliable assay system to identify therapeutic agents for treating diseases and conditions such as obesity, diabetes, and hyperinsulinemia. Therefore, Applicants respectfully submit that a variety of real-life utilities, such as treatments for glucose

uptake related diseases, including obesity and diabetes, are envisioned for PRO1303 based on the glucose/FFA uptake assay results disclosed herein.

The Examiner asserts that "the specification does not indicate which asserted utilities correspond specifically to glucose uptake stimulation as opposed to glucose uptake inhibition." (Pages 2-3 of the instant Office Action). As discussed above, it was known in the art at the time of filing that agents which increased glucose uptake, such as troglitazone and pioglitazone, were useful in the treatment of diabetes. Treatment with vanadium salts, another agent which increased glucose uptake, was shown to lower glucose levels in hyperglycemic rats. Diabetes, hyperglycemia, and obesity were know at the time of filing to be closely linked conditions (see, for example, Sandouk, page 352). Thus one of skill in the art would have understood that stimulators of glucose uptake would be useful in the treatment of diabetes, obesity, and hyperglycemia.

The Examiner asserts that "the specification does not indicate what, if any, of the utilities set forth correspond to stimulation of FFA uptake." (Page 3 of the instant Office Action). The Examiner further asserts that stimulation of glucose and/or FFA uptake is actually "three very different activities (stimulation of glucose uptake only, stimulation of FFA uptake only, and stimulation of uptake of both)." (Page 3 of the instant Office Action). Finally, the Examiner asserts that "it is unclear how increasing uptake of FFA into adipocytes would treat obesity (or thus diabetes)." (Page 3 of the instant Office Action). The Examiner cites Fabris *et al.* as teaching that "FFA-induced insulin resistance saves scarce glucose for central nervous system requirements, but this becomes counterproductive in obesity because it inhibits glucose utilization when there is no need to save it." (Page 3 of the instant Office Action).

Applicants respectfully point out that it was well known in the art at the time of filing that both glucose and FFA levels were associated with diabetes, obesity, and hyperinsulinemia. In fact, FFA levels are one of the factors which regulate glucose uptake. Applicants respectfully direct the Examiner's attention to the later discussion in Fabris *et al.*, where the authors make clear that FFA-induced insulin resistance is a result of high circulating FFA levels (page 604, col. 2). As the portion of Fabris *et al.* cited by the Examiner explains, this resistance leads to less

utilization of glucose, which contributes to the development of obesity or diabetes. Thus Fabris *et al.* supports Applicants' assertion that decreasing circulating FFA levels, by increasing FFA uptake into adipocytes, will run counter to this trend and help in the treatment of obesity or diabetes. In addition, Santomauro *et al.* (made of record by the Examiner in the Office Action mailed April 5, 2005) demonstrated that "lowering of elevated plasma FFA levels can reduce insulin resistance/hyperinsulinemia and improve oral glucose tolerance in lean and obese nondiabetic subjects and in obese patients with type 2 diabetes" (Abstract). Thus it is clear that agents which decrease circulating FFA levels are effective in the treatment of diseases such as obesity and diabetes.

It was further known in the art at the time of filing that antidiabetic agents such as the thiazolidinediones (including troglitazone and pioglitazone) discussed above as agents which increase glucose uptake, also increase FFA uptake by adipocytes. For example, Frohnert *et al.* (J. Biol. Chem. 272:3970-3977 (1999); copy enclosed) found that troglitazone stimulates increased expression of fatty acid transport protein (FATP) in adipocytes by 3-fold (page 3974, col. 1; Fig. 5). Troglitazone was also found to significantly increase uptake of fatty acids by adipocytes (page 3974, col. 2 and Fig. 8). The authors conclude that the antidiabetic effects of troglitazone may be due to improved fatty acid uptake by adipocytes (page 3976, col. 2). Similarly, Martin *et al.* (J. Biol. Chem. 272:28210-28217 (1997); copy enclosed) found that the antidiabetic thiazolidinedione BRL 49653 "resulted in a significant induction of adipose tissue FATP (7-fold) ... mRNA levels" (page 28213, col. 2). The induction of FATP mRNA also resulted in increased FFA uptake into adipocytes (page 28214, col. 2). The authors conclude that "thiazolidinedione antidiabetic agents seem to favor adipocyte-specific FA uptake relative to muscle, perhaps underlying in part the beneficial effects of these agents on insulin-mediated glucose dispersal" (Abstract). Thus the art at the time of filing demonstrated that agents which increased FFA uptake by adipocytes were useful in the treatment of diabetes.

The Examiner asserts that Applicants' argument "does not take into account the effect of the increased levels of FFA in adipocytes and the probable contribution this would have to

obesity" and cites Berk *et al.* as teaching that "increased FFA uptake in adipocytes is present in genetically obese rats." (Page 6 of the instant Office Action).

Applicants respectfully submit that Berk *et al.* admit that "[t]he relevance of this study to human obesity remains to be established" (page 8834, col. 2). Fabris *et al.* explain that FFA-induced insulin resistance primarily affects muscle, leading to the shift of glucose from utilization in muscle to storage in adipocytes observed in obesity. Fabris *et al.* make clear, however, that FFA-induced insulin resistance is a result of high circulating FFA levels, thus supporting Applicants' assertion that decreasing circulating FFA levels, by increasing FFA uptake into adipocytes, will run counter to this trend and prevent the selective partitioning of glucose into adipocytes in place of muscle that leads to obesity. Santomauro *et al.* confirm this assertion by demonstrating that "lowering of elevated plasma FFA levels can reduce insulin resistance/hyperinsulinemia and improve oral glucose tolerance in lean and obese nondiabetic subjects and in obese patients with type 2 diabetes" (Abstract). Furthermore, as discussed above, it was known in the art at the time of filing, as evidenced by Frohnert *et al.* and Martin *et al.*, that known antidiabetic agents such as the thiazolidinediones (including troglitazone and pioglitazone) increase FFA uptake by adipocytes. Thus the art is clear that agents which increase FFA uptake by adipocytes are in fact useful in the treatment of diabetes.

The Examiner further asserts that "the observed differences do not appear to be statistically significant, and the cutoff points appear to be arbitrary and there is no scientific basis for them." (Page 3 of the instant Office Action). In support of this assertion, the Examiner cites Santomauro *et al.* Santomauro *et al.* teach that 56.5% decreases in FFA levels are statistically significant and correlated with physiological improvement. The Examiner asserts that "it is not clear from either the prior art or the specification whether 50% decreases are useful." (Page 3 of the instant Office Action). Applicants respectfully submit that the difference between 56.5% and 50% is not large. Applicants further respectfully point out that the treatments in Santamauro *et al.* resulted in lowering FFA levels to below normal. One of skill in the art would expect that lowering FFA levels to only normal levels (requiring a decrease of only 41-44%) would also be useful.

The Examiner asserts that "Santomauro *et al.* discloses results of *in vivo* levels of FFA, not *in vitro* levels as disclosed in the specification. The two are not fair comparisons." (Page 7 of the instant Office Action). The Examiner further asserts that "the observation that 56.5% decreases in circulating FFAs is significant and correlated with physiological improvements does not mean that a doubling of uptake of FFAs by adipocytes will lead to the same decreases in FFAs." (Page 4 of the instant Office Action). Applicants respectfully note that no evidence is provided for this assertion. To the contrary, the art indicates that *in vitro* uptake experiments with rat adipocytes actually underpredict the effects of treatment *in vivo*. For example, Goldwaser *et al.* demonstrated that vanadium ions increased glucose uptake in rat adipocytes *in vitro* by 4-5 fold, but lowered blood glucose levels in hyperglycemic rats *in vivo* by 5-7 fold (see Abstract). Thus the Office Action provides no evidence that the disclosed *in vitro* results would not correlate with *in vivo* results, as has been previously demonstrated in the art.

The Examiner asserts that "[w]hile other groups may have also done the same glucose/FFA assay *in vitro* using other compounds, and shown upon further research that their compound is useful to treat disease, this does not prove anything regarding the instant compound." (Page 5 of the instant Office Action).

Applicants respectfully submit that this is an inappropriate standard for the assessment of *in vitro* data. With respect to disclosure of the results of *in vitro* assays, the M.P.E.P. states that "if the art is such that a particular model is recognized as correlating to a specific condition, **then it should be accepted as correlating** unless the examiner has evidence that the model does not correlate. Even with such evidence, the examiner must weigh the evidence for and against correlation and decide whether one skilled in the art would accept the model as reasonably correlating to the condition. *In re Brana*, 51 F.3d 1560, 1566, 34 USPQ2d 1436, 1441 (Fed. Cir. 1995)."[1] (Emphasis added).

The M.P.E.P. also makes clear that the burden of proof is on the examiner to demonstrate lack of correlation for an *in vitro* model. "Since the initial burden is on the examiner to give

[1] M.P.E.P. § 2164.02.

reasons for the lack of enablement, the examiner must also give reasons for a conclusion of lack of correlation for an *in vitro* or *in vivo* animal model example."[2] A rigorous or an invariable exact correlation is not required, as stated in *Cross v. Iizuka,*[3] wherein the court stated that "based upon the relevant evidence as a whole, there is a reasonable correlation between the disclosed *in vitro* utility and an *in vivo* activity, and therefore a rigorous correlation is not necessary where the disclosure of pharmacological activity is reasonable based upon the probative evidence."[4]

Applicants respectfully submit that, as discussed above, it is well established in the art that a positive result as an stimulator in the *in vitro* adipocyte glucose/FFA uptake assay is reasonably correlated to use as a therapeutic compound for the treatment of disorders such as diabetes. See, for example, Tafûri, Sandouk *et al.*, and Goldwasser *et al.* (all made of record in Applicants' IDS filed July 5, 2005), as well as Frohnert *et al.* and Martin *et al.*, submitted herein. Thus, by providing the experimental results demonstrating that PRO1303 tested positive as a stimulator in the adipocyte glucose/FFA uptake assay, the instant specification has provided all that is required to demonstrate utility for the claimed PRO1303 polypeptides.

Finally, the Examiner asserts that the results of the assay disclosed in Example 149 "does not provide the utility **in currently available form** of treating humans for any condition listed" because "problems such as toxicity, method of delivery to appropriate cell types, stability in the bloodstream, dosage, etc. which would affect the utility in effective treatment of complex conditions such as obesity and diabetes have note been solved, or even contemplated to be approached, by the disclosure." (Page 4 of the instant Office Action; emphasis in original).

Applicants respectfully submit that it is well established that evidence of treatment in humans is not required to demonstrate utility. M.P.E.P. §2107.03 (III) states that:

> "If reasonably correlated to the particular therapeutic or pharmacological utility, data generated using *in vitro* assays, or from testing in an animal model or a

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[2] M.P.E.P. § 2164.02.

[3] *Cross v. Iizuka*, 753 F.2d 1040, 224 U.S.P.Q. 739 (Fed. Cir. 1985).

[4] *Id* at 1050, 224 U.S.P.Q. at 747.

combination thereof almost invariably will be sufficient to establish therapeutic or pharmacological utility for a compound, composition or process."

Thus, the legal standard recognizes that *in vitro* or animal model data is acceptable to establish utility as long as the data is "reasonably correlated" to the pharmacological utility described.

Applicants further respectfully submit that enablement "is not precluded even if some experimentation is necessary, although the amount of experimentation needed must not be unduly extensive."[5] As the M.P.E.P. states, "[t]he fact that experimentation may be complex does not necessarily make it undue, if the art typically engages in such experimentation."[6] The M.P.E.P. further explains that "If a statement of utility in the specification contains within it a connotation of how to use, and/or the art recognizes that standard modes of administration are known and contemplated, 35 U.S.C. 112 is satisfied."[7] Applicants note that the specification clearly indicates that the claimed polypeptides are useful in the treatment of disorders associated with abnormal glucose metabolism, such as obesity, diabetes, and hyperinsulinemia. The use of molecules that stimulate adipocyte glucose/FFA uptake in the treatment of such disorders is well known in the art, as indicated by Tafuri, Sandouk *et al.*, and Goldwasser *et al.* (all made of record in Applicants' IDS filed July 5, 2005), as well as Frohnert *et al.* and Martin *et al.*, submitted herein. Thus any further experimentation required to determine, for example, the particular dosage or method of administration of PRO1303 would not be considered undue.[8]

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[5] *Hybritech Inc. v. Monoclonal Antibodies, Inc.*, 802 F.2d 1367, 231 U.S.P.Q. 81 (Fed. Cir. 1986), *cert denied*, 480 U.S. 947(1987).

[6]. M.P.E.P. 2164.01 citing *In re Certain Limited-charge Cell Culture Microcarriers*, 221 U.S.P.Q. 1165, 1174 (Int'l Trade Comm'n 1983), *aff' sub nom. Massachusetts Institute of Technology v. A.B. Fortia* 774 F.2d 1104, 227 U.S.P.Q. 428 (Fed. Cir. 1985).

[7]. M.P.E.P. 2165.01(c) citing *In re Johnson*, 282 F.2d 370, 373, 127 U.S.P.Q. 216, 219 (CCPA 1960); *In re Hitchings*, 342 F.2d 80, 87, 144 U.S.P.Q. 637, 643 (CCPA 1965); and *In re Brana*, 51 F.2d 1560, 1566, 34 U.S.P.Q.2d 1437, 1441 (Fed. Cir. 1993).

[8]. M.P.E.P. 2165.01(c).

Finally, Applicants respectfully remind the Examiner that in explaining the "substantial utility" standard, M.P.E.P. §2107.01 cautions that Office personnel must be careful not to interpret the phrase "immediate benefit to the public" or similar formulations used in certain court decisions to mean that products or services based on the claimed invention must be "currently available" to the public in order to satisfy the utility requirement. "Rather, any reasonable use that an applicant has identified for the invention that can be viewed as providing a public benefit should be accepted as sufficient, at least with regard to defining a "'substantial'" utility.'" (M.P.E.P. §2107.01) Indeed, the Guidelines for Examination of Applications for Compliance With the Utility Requirement, (M.P.E.P. §2107 II(B)(1)) gives the following instruction to patent examiners: "If the applicant has asserted that the claimed invention is useful for any particular practical purpose . . . and the assertion would be considered credible by a person of ordinary skill in the art, do not impose a rejection based on lack of utility."

As discussed above, at the time of the effective filing date of the instant application, it was well known in the art that increasing glucose uptake by adipocyte cells is a hallmark of a number of therapeutically effective agents, such as troglitazone and pioglitazone. The art has also shown that agents which decrease circulating FFA levels are also useful in the treatment of disorders such as diabetes, hyperglycemia, and obesity. One of ordinary skill in the art would therefore find it more likely than not that an agent which increases uptake of glucose and/or FFA by adipocytes would also be useful in the treatment of disorders such as diabetes, hyperglycemia, and obesity. Accordingly, a *prima facie* showing of lack of utility has not been made in this instance and the burden to provide further evidence of utility has not shifted to Applicants.

The results of the gene amplification assay also provide utility for the PRO1303 polypeptide

Applicants respectfully note that they are required to disclose only a single patentable utility for their claimed invention. Nonetheless, in addition to the utilities discussed above in the treatment of disorders such as obesity, diabetes, and hyper- or hypo-insulinemia, Applicants respectfully submit that the gene amplification data also demonstrates patentable utility for the

PRO1303 polypeptide and the claimed antibodies that bind it. The gene amplification data for the gene encoding the PRO1303 polypeptide is clearly disclosed in the instant specification under Example 143.

As discussed in Applicants' previous Responses and Appeal Brief, the specification discloses that the nucleic acids encoding PRO1303 had ΔCt value of > 1.0, which is a **more than 2-fold increase**, for primary lung tumors LT13, LT15, LT16; for lung cell line A549; and for the primary colon tumor CT16. PRO1303 showed approximately 1.13 to 1.42 ΔCt units which corresponds to $2^{1.13}$ to $2^{1.42}$- fold amplification or 2.19 to 2.68 fold amplification in primary lung tumors LT13, LT15, LT16; for lung cell line A549; and for the primary colon tumor CT16. (See Table 8 of the specification). Accordingly, the present specification clearly discloses strong evidence that the gene encoding the PRO1303 polypeptide is significantly amplified in a number of lung and colon tumors. Thus one of ordinary skill in the art would find it credible that the PRO1555 polypeptide and the claimed antibodies that bind it have utility as diagnostic markers of lung and colon tumors.

A prima facie case of lack of utility has not been established

The Examiner asserts that the disclosed gene amplification data has "no bearing on the utility of the encoded polypeptides" because allegedly "amplified genomic DNA would have to correlated with amplified mRNA, which in turn would have to correlate with amplified polypeptide levels. The art discloses that such correlations cannot be presumed." (Pages 7-8 of the instant Office Action). In support of this assertion, the Examiner first refers to the previously cited references by Pennica *et al.* and Konopka *et al.*

As discussed in Applicants' Brief filed May 2, 2006, the evidentiary standard to be used throughout *ex parte* examination of a patent application is a preponderance of the totality of the evidence under consideration. Accordingly, Applicants submit that in order to overcome the presumption of truth that an assertion of utility by the applicant enjoys, the Examiner must establish that **it is more likely than not** that one of ordinary skill in the art would doubt the truth of the statement of utility. **The standard is not absolute certainty.** The law requires only that

one skilled in the art should accept that such a correlation is **more likely than not to exist**. Applicants submit that the references cited by the PTO are either irrelevant, or actually offer support for Applicants' position, as discussed below. Even if the PTO has met its initial burden, Applicants have submitted enough rebuttal evidence such that it is **more likely than not** that a person of skill in the art would be convinced, **to a reasonable probability,** that the asserted utility is true.

Pennica *et al.* and Konopka *et al.*

Applicants submit that Pennica *et al.* does not show a lack of correlation between gene (DNA) amplification and mRNA levels. According to Pennica *et al.*, "WISP-1 gene amplification in human colon tumors showed a correlation between DNA amplification and over-expression, whereas overexpression of WISP-3 RNA was seen in the absence of DNA amplification. In contrast, WISP-2 DNA was amplified in colon tumors, but its mRNA expression was significantly reduced in the majority of tumors compared with expression in normal colonic mucosa from the same patient" (Abstract). From this, the Examiner correctly concludes that increased copy number does not *necessarily* result in increased polypeptide expression. The standard, however, is not absolute certainty.

In fact, as noted even in Pennica *et al.*, "[a]n analysis of *WISP*-1 gene amplification and expression in human colon tumors *showed a correlation between DNA amplification and over-expression...*" (Pennica *et al.*, page14722, left column, first full paragraph, emphasis added). Thus the findings of Pennica *et al.* with respect to WISP-1 support Applicants' arguments. In the case of WISP-3, the authors report that there was no change in the DNA copy number, but there was a change in mRNA levels. This apparent lack of correlation between DNA and mRNA levels is not contrary to Applicants' assertion that a change in DNA copy number generally leads to a change in mRNA level. Applicants are not attempting to predict the DNA copy number based on changes in mRNA level, and Applicants have not asserted that the only means for changing the level of mRNA is to change the DNA copy number. Therefore a change in mRNA without a change in DNA copy number is not contrary to Applicants' assertions.

The fact that the single WISP-2 gene did not show the expected correlation of gene amplification with the level of mRNA/protein expression does not establish that it is more likely than not, in general, that such correlation does not exist. The Examiner has not shown whether the lack or correlation observed for the WISP-2 gene is typical, or is merely a discrepancy, an exception to the rule of correlation. More importantly, the teaching of Pennica *et al.* is specific to *WISP* genes. Pennica *et al.* has no teaching whatsoever about the correlation of gene amplification and protein expression in general.

The Examiner argues that Pennica *et al* is relevant even though it is limited to only one gene family because "the instant application concerns only one gene as well." (Page 14 of the instant Office Action). Applicants respectfully disagree. The test is whether it is more likely than not that gene amplification results in overexpression of the corresponding mRNA and protein. In order to meet that standard, the Examiner must provide evidence that it is more likely than not that gene amplification does not result in mRNA or protein overexpression. Providing the single example of the WISP-2 gene does not suffice to meet this burden.

Applicants next respectfully submit that, contrary to the PTO's assertions, Konopka *et al.* **supports** Applicants' position that mRNA levels correlate with protein levels. Konopka *et al.* states that "the 8-kb mRNA that encodes $P210^{c\text{-}abl}$ was detected at a 10-fold higher level in SK-CML7bt-333 (Fig. 3A, +) than in SK-CML16Bt-1 (B, +), which **correlated** with the relative level of $P210^{c\text{-}abl}$ detected in each cell line. Analysis of additional cell lines demonstrated that the level of 8-kb mRNA **directly correlated** with the level of $P210^{c\text{-}abl}$ (Table 1)" (page 4050, col. 2, emphasis added).

Nor does Konopka *et al.* support the PTO's position that DNA amplification is not correlated with mRNA or protein overexpression. Konopka *et al.* show only that, of the cell lines known to have increased abl protein expression, only one had amplification of the abl gene (page 4051, col. 1). This result proves only that increased mRNA and protein expression levels can result from causes other than gene amplification. Konopka *et al.* do not demonstrate that when gene amplification does occur, it does not result in increased mRNA and protein

expression levels, particularly given that the cell line with amplification of the abl gene did show increased abl mRNA and protein expression levels.

The Examiner next asserts that "even if increased mRNA levels could be established for PRO1303, it does not follow that polypeptide levels would also be amplified." (Page 8 of the instant Office Action). In support of this assertion, the Examiner refers to newly cited references by Chen *et al.*, Hu *et al.*, and LaBaer.

Chen *et al.*

The Examiner cites Chen *et al.* as allegedly disclosing that "[o]nly 17% of 165 protein spots or 21% of the genes had a significant correlation between protein and mRNA expression levels" and that "it is not possible to predict overall protein expression levels based upon average mRNA abundance in lung cancer samples." (Page 8 of the instant Office Action).

First, Applicants note that proteins selected for study by Chen *et al.* were those detectable by staining of 2D gels. As noted in, for example, Haynes *et al.*, cited by the Examiner, there are problems with selecting proteins detectable by 2D gels. "It is apparent that without prior enrichment only a relatively small and highly selected population of long-lived, highly expressed proteins is observed. There are many more proteins in a given cell which are not visualized by such methods. Frequently it is the low abundance proteins that execute key regulatory functions" (page 1870, col. 1). Thus, Chen *et al.*, by selecting proteins detectable by staining of 2D gels, are likely to have excluded from their analysis many of the proteins most likely to be significant as cancer markers.

Secondly, Chen *et al.* looked at expression levels across a set of samples including a large number of tumor samples (76) along with a much smaller number of normal samples (9). The tumor samples were taken from stage 1 and stage III lung adenocarcinomas, which were classified as bronchoaveolar, bronchial derived or both bronchial and bronchoaveolar derived. Accordingly, the tissues examined were from different tissues in different stages of normal or cancerous growth. The authors determined the relationship between mRNA and protein expression by using the average expression values for all samples. The average value for each protein or mRNA was generated using all 85 lung tissue samples. This resulted in negative

normalized protein values in some cases. Further, the authors chose an arbitrary threshold of 0.115 for the correlation to be considered significant. Accordingly, the Chen paper does not account for different expression in different tissues or different stages of cancer.

Thirdly, no attempt was made to compare expression levels in normal versus tumor samples, and in fact the authors concede that they had too few normal samples for meaningful analysis (page 310, col. 2). As a result, the analysis in the Chen paper shows only that a number of randomly selected proteins have varying degrees of correlation between mRNA and protein expression levels within a set of different lung adenocarcinoma samples. The Chen paper does not address the issue of whether increased mRNA levels in the tumor samples taken together as one group, as compared to the normal samples as a group, correlated with increased protein levels in tumorous versus normal tissue. Accordingly, the results presented in the Chen paper are not applicable to the application at issue.

The correct test of utility is whether the utility is "more likely than not." In the case of the Chen reference, even if the analysis presented is correct (which is disputed), a review of the correlation coefficient data presented in the Chen *et al.* paper indicates that it is more likely than not that increased mRNA expression correlates with increased protein expression. A review of Table 1, which lists 66 genes [the paper incorrectly states there are 69 genes listed] for which only one protein isoform is expressed, shows that 40 genes out of 66 had a positive correlation between mRNA expression and protein expression. This clearly meets the test of "more likely than not." Similarly, in Table II , 30 genes with multiple isoforms [again the paper incorrectly states there are 29] were presented. In this case, for 22 genes out of 30, at least one isoform showed a positive correlation between mRNA expression and protein expression. Furthermore, 12 genes out of 29 showed a strong positive correlation [as determined by the authors] for at least one isoform. No genes showed a significant negative correlation. It is not surprising that not all isoforms are positively correlated with mRNA expression. Certain isoforms are likely non-functional proteins. Thus, Table II also provides that it is more likely than not that protein levels will correlate with mRNA expression levels.

The same authors in Chen *et al.*, published a later paper, Beer *et al.*, Nature Medicine 8(8) 816-824 (2002) (copy enclosed) which described gene expression of genes in adenocarcinomas and compared that to protein expression. In this paper they report that "these results suggest that the oligonucleotide microarrays provided reliable measures of gene expression" (page 817). The authors also state, "these studies indicate that many of the genes identified using gene expression profiles are likely relevant to lung adenocarcinoma." Clearly the authors of the Chen paper agree that microarrays provide a reliable measure of the expression levels of the gene and can be used to identify genes whose overexpression is associated with tumors.

Hu *et al.* and LaBaer

The Examiner cites Hu *et al.* to the effect that genes displaying a 5-fold change or less in mRNA expression in tumors compared to normal showed no evidence of a correlation between altered gene expression and a known role in the disease. However, among genes with a 10-fold or more change in expression level, there was a strong and significant correlation between expression level and a published role in the disease. (Page 9 of the instant Office Action).

Applicants respectfully submit that the statistical analysis by Hu *et al.* is not a reliable standard because the frequency of citation only reflects the current research interest in a molecule, not the true biological function of the molecule. Indeed, the authors acknowledge that "[r]elationships established by frequency of co-citation do not necessarily represent a true biological link." (See page 411, right column). One would expect that genes with the greatest change in expression in a disease would be the first targets of research, and therefore have the strongest known relationship to the disease as measured by the number of publications reporting a connection with the disease. The correlation reported in Hu only indicates that the greater the change in expression level, the more likely it is that there is a published or known role for the gene in the disease, as found by their automated literature-mining software. Thus, Hu's results merely reflect a bias in the literature toward studying the most prominent targets, and say nothing regarding the ability of a gene that is 2-fold or more differentially expressed in tumors to serve as a disease marker.

Even assuming that Hu *et al.* provide evidence to support a true relationship, the conclusion in Hu *et al.* only applies to a specific type of breast tumor (estrogen receptor (ER)-positive breast tumor) and can not be generalized as a principle governing microarray study of breast cancer in general, let alone the various other types of cancer genes in general. In fact, even Hu *et al* admit that., "[i]t is likely that this threshold will change depending on the disease as well as the experiment. Interestingly, the observed correlation was only found among ER-positive (breast) tumors not ER-negative tumors." (See page 412, left column). Therefore, based on these findings, the authors add, "[t]his may reflect a bias in the literature to study the more prevalent type of tumor in the population. Furthermore, this emphasizes that caution must be taken when interpreting experiments that may contain subpopulations that behave very differently." (See page 412, left column; emphasis added).

Furthermore, Hu *et al.* did not look for a correlation between changes in mRNA and changes in protein levels, and therefore their results are not contrary to Applicants' assertion that there is a correlation between the two. Applicants are not relying on any "biological role" that the PRO1303 polypeptide has in cancer for its asserted utility. Instead, Applicants are relying on the overexpression of PRO1303 in certain tumors compared to their normal tissue counterparts. Nowhere in Hu does it say that a lack of correlation in their study means that genes with a less than five-fold change in level of expression in cancer cannot serve as a diagnostic marker of cancer.

The Examiner next cites LaBaer to the effect that "reports of RNA or protein changes of as little as two-fold are not uncommon, and although changes of this magnitude may turn out to be important, most are attributable to disease-independent differences between the samples." (Page 9 of the instant Office Action).

Applicants respectfully point out that LaBaer is an unreviewed letter to the editor by an author of the Hu *et al.* article describing the automated literature searching tool used in the Hu *et al.* reference discussed above. LaBaer provides no further evidence than that provided in Hu, and provides no evidence whatsoever to support the conclusion that the results of Hu are applicable to the diagnostic utility of differentially expressed genes. Importantly, like the Hu reference,

LaBaer does not consider or offer any discussion of whether there is a correlation between changes in mRNA levels and changes in the level of the encoded protein. In addition, LaBaer's conclusions regarding disease-independent differences between samples are not applicable in the instant case where normal human tissue and human tumor tissue samples were compared. Accordingly, LaBaer suffers from the same defects discussed above with respect to Hu *et al.*

The Examiner futher asserts that "[t]he art also shows that mRNA (transcript) levels do not correlate with polypeptide levels in normal tissues." (Page 9 of the instant Office Action). In support of this assertion, the Examiner cites articles by Haynes *et al.*, Gygi *et al.*, Lian *et al.*, Fessler *et al.*, and Greenbaum *et al.*

Haynes *et al.*

The Examiner asserts that the Haynes *et al.* reference found "no strong correlation between polypeptide and transcript levels." (Page 9 of the instant Office Action). Applicants respectfully point out that, on the contrary, Haynes *et al.* never indicate that the correlation between mRNA and protein levels does not exist. Haynes *et al.* only state that "protein levels cannot be *accurately* predicted from the level of the corresponding mRNA transcript" (See page 1863, under Section 2.1, last line, emphasis added). This result is expected, since there are many factors that determine translation efficiency for a given transcript, or the half-life of the encoded protein. Not surprisingly, Haynes *et al.* concluded that protein levels cannot always be accurately predicted from the level of the corresponding mRNA transcript in a single cellular stage or type when looking at the level of transcripts across different genes.

Importantly, Haynes *et al.* did not say that for a single gene, a change in the level of mRNA transcript is not positively correlated with a change in the level of protein expression. Applicants have asserted that increasing the level of mRNA for a particular gene leads to a corresponding increase for the encoded protein. Haynes *et al.* did not study this issue and says absolutely nothing about it. One cannot look at the level of mRNA across several different genes to investigate whether a change in the level of mRNA for a particular gene leads to a change in the level of protein for that gene. Therefore, Haynes *et al.* is not inconsistent with or

contradictory to the utility of the instant claims, and offers no support for the PTO's rejection of Applicants' asserted utility.

Furthermore, Applicants note that contrary to the Examiner's statement, Haynes teaches that "*there was a general trend* but no strong correlation between protein [expression] and transcript levels" (See page 1863, under Section 2.1, emphasis added). For example, in Figure 1, there is a positive correlation between mRNA and protein amongst *most* of the 80 yeast proteins studied but the correlation is not linear, hence the authors suggest that one cannot *accurately* predict protein levels from mRNA levels. In fact, very few data points deviated or scattered away from the expected normal or showed a lack of correlation between mRNA: protein levels. Thus, the Haynes data meets the "more likely than not standard" and shows that a positive correlation exists between mRNA and protein. Therefore, Applicants submit that the Examiner's rejection is based on a misrepresentation of the scientific data presented in Haynes *et al.*

Haynes *et al.* may teach that protein levels cannot be "accurately predicted" from mRNA levels in the sense that the exact numerical amounts of protein present in a tissue cannot be determined based upon mRNA levels. Applicants respectfully submit that the PTO's emphasis on the need to "accurately predict" protein levels based on mRNA levels misses the point. The asserted utility for the claimed polypeptides is in the diagnosis of cancer. What is relevant to use as a cancer diagnostic is relative levels of gene or protein expression, not absolute values, that is, that the gene or protein is differentially expressed in tumors as compared to normal tissues. Applicants need only show that there is a correlation between mRNA and protein levels, such that mRNA overexpression generally predict protein overexpression. A showing that mRNA levels can be used to "accurately predict" the precise levels of protein expression is not required.

Gygi *et al.*

The Examiner further cites Gygi *et al.*, a study on which the Haynes references is based. Like Haynes, the Gygi reference looked at levels of mRNA at the same growth phase across different genes, not changes in mRNA levels for a single gene. Thus, when Gygi *et al.* state that "the correlation between mRNA and protein levels was insufficient to predict protein expression levels from quantitative mRNA data," the authors are referring to correlations between constant

levels of mRNA and protein at the same growth phase across different genes, not a correlation between a change in mRNA level and a change in protein level for the same gene and corresponding protein. Therefore, for the same reasons that Haynes is not relevant to Applicants' asserted utility, Gygi likewise offers no support for the PTO's rejection of Applicants' asserted utility.

Furthermore, Applicants note that contrary to the Examiner's statement, the Gygi data indicate **a general trend** of correlation between protein [expression] and transcript levels (Emphasis added). For example, as shown in Figure 5, an mRNA abundance of **250-300** copies /cell correlates with a protein abundance of **500-1000** x 10^3 copies/cell. An mRNA abundance of **100-200** copies/cell correlates with a protein abundance of **250-500** x 10^3 copies/cell (emphasis added). Therefore, high levels of mRNA **generally** correlate with high levels of proteins. In fact, most data points in Figure 5 did not deviate or scatter away from the general trend of correlation. Thus, the Gygi data meets the "more likely than not standard" and shows that a positive correlation exists between mRNA and protein. Therefore, Applicants submit that the Examiner's rejection is based on a misrepresentation of the scientific data presented in Gygi *et al.*

Applicants further respectfully submit that Futcher *et al.* (Mol. Cell. Biol. 19:7357-7368 (1999); copy enclosed as Exhibit 13) also analyzed the yeast proteome using 2D gel electrophoresis, gathering quantitative data on protein abundance for about 1,400 spots. This data was compared to mRNA abundance for each gene as determined both by SAGE (serial analysis of gene expression) and by hybridization of cRNA to oligonucleotide arrays. The authors concluded that "**several statistical methods show a strong and significant correlation between mRNA abundance and protein abundance**" (page 7360, col. 2; emphasis added).

The authors note that Gygi *et al.* completed a similar study that generated broadly similar data, but reached different conclusions. Futcher *et al.* point out that "the different conclusions are also partly due to different methods of statistical analysis, and to real differences in data." Futcher *et al.* note that Gygi *et al.* used the Pearson product-moment correlation coefficient (r_p) and point out that "a calculation of r_p is inappropriate" because the mRNA and protein

abundances are not normally distributed (page 7367, col. 1). In contrast, Futcher *et al.* used two different statistical approaches to determining the correlation between mRNA and protein abundances. First, they used the Spearman rank correlation coefficient (r_s), an nonparametric statistic that does not require the data to be normally distributed. Using the r_s, the authors found that mRNA abundance was well correlated with protein abundance ($r_s = 0.74$). Applying this statistical approach to the data of Gygi *et al.* **also** resulted in a good correlation ($r_s = 0.59$), although the correlation was not quite as strong as for the Futcher *et al.* data. In a second approach, Futcher *et al.* transformed the mRNA and protein data to forms where they were normally distributed, in order to allow calculation of an r_p. Two types of transformation (Box-Cox and logarithmic) were used, and **both** resulted in good correlations between mRNA and protein abundance for Futcher *et al.*'s data.

Futcher *et al.* also note that the two studies used different methods of measuring protein abundance. Gygi *et al.* cut spots out of each gel and measured the radiation in each spot by scintillation counting, whereas Futcher *et al.* used phosphorimaging of intact gels coupled to image analysis. Futcher *et al.* point out that Gygi *et al.* may have systematically overestimated the amount of the lowest-abundance proteins, because of the difficulty in accurately cutting out very small spots from the gel, and because of difficulties in background subtraction for small, weak spots.

In addition, Futcher *et al.* note that they used both SAGE data and RNA hybridization data to determine mRNA abundances, which is most helpful to accurately measure the least abundant mRNAs. As a result, while the Futcher data set "maintains a good correlation between mRNA and protein abundance even at low protein abundance" (page 7367, col. 2), the Gygi data shows a strong correlation for the most abundant proteins, but a poor correlation for the least abundant proteins in their data set. Futcher *et al.* conclude that "**the poor correlation of protein to mRNA for the nonabundant proteins of Gygi *et al.* may reflect difficulty in accurately measuring these nonabundant proteins and mRNAs, rather than indicating a truly poor correlation *in vivo***" (page 7367, col. 2; emphasis added). Thus while these lowest abundant

proteins do show a poor correlation, this is almost certainly due to the less accurate methods used to measure the abundance of these proteins, and **not** to any actual lack of correlation.

Lian *et al.*

The PTO cites Lian *et al.* for the statement that there is a poor correlation between mRNA expression and protein abundance in mouse cells, and therefore it may be difficult to extrapolate directly from individual mRNA changes to corresponding ones in protein levels. (Page 10 of the instant Office Action).

In Lian *et al.*, the authors looked at the mRNA and protein levels of genes in a derived promyelocytic mouse cell-line during differentiation of the cells from a promyelocytic stage of development to mature neutrophils following treatment with retinoic acid. The level of mRNA expression was measured using 3'-end differential display (DD) and oligonucleotide chip array hybridization to examine the expression of genes at 0, 24, 48 and 72 hours after treatment with retinoic acid. Protein levels were qualitatively assessed at 0 and 72 hours after retinoic acid treatment following 2-dimensional gel electrophoresis.

Lian *et al.* report that they were able to identify 28 proteins which they considered differentially expressed (page 521). Of those 28, only 18 had corresponding gene expression information, and only 13 had measurable levels of mRNA expression (page 521, Table 6). The authors then compared the qualitative protein level from the 2-D electrophoresis gel to the corresponding mRNA level, and reported that only 4 genes of the 18 present in the database had expression levels which were consistent with protein levels (page 521, col. 1). The authors note that "[n]one of these was on the list of genes that were differentially expressed significantly (5-fold or greater change by array or 2-fold or greater change by DD)" (page 521; emphasis added). Based on these data, the authors conclude "[f]or protein levels based on estimated intensity of Coomassie dye staining in 2DE, there was poor correlation between changes in mRNA levels and estimated protein levels" (page 522, col. 2).

The authors themselves admit that there are a number of problems with the data presented in this reference. At page 520 of this article, the authors explicitly express their concerns by stating that "[t]hese data must be considered with several caveats: membrane and other

hydrophobic proteins and very basic proteins are not well displayed by the standard 2DE approach, and proteins presented at low level will be missed. In addition, to simplify MS analysis, we used a Coomassie dye stain rather than silver to visualize proteins, and this decreased the sensitivity of detection of minor proteins." (emphasis added). It is known in the art that Coomassie dye stain is a very insensitive method of measuring protein. This suggests that the authors relied on a very insensitive measurement of the proteins studied. The conclusions based on such measurements can hardly be accurate or generally applicable. In particular, the total number of proteins examined by Lian *et al.* was only 50 (page 520, col. 2), as compared to the approximately 7000 genes for which mRNA levels were measured (page 515, col. 1). Thus the conclusions are based on a very small and atypical set of proteins.

Applicants also emphasize that Applicants are asserting that a measurable change in mRNA level generally leads to a corresponding change in the level of protein expression, not that changes in protein level can be used to predict changes in mRNA level. As discussed above, Lian *et al.* did not take genes which showed significant mRNA changes and check the corresponding protein levels. Instead, the authors looked at a small and unrepresentative number of proteins, and checked the corresponding mRNA levels. Based on the authors' criteria, mRNA levels were significantly changed if they were at least 5-fold different when measured using a microchip array, or 2-fold different when using the more sensitive 3'-end differential display (DD). Of the 28 proteins listed in Table 6, only one has an mRNA level measured by microarray which is differentially expressed according to the authors (spot 7: melanoma X-actin, for which mRNA changed from 2539 to 341.3, and protein changed from 1 to 3). None of the other mRNAs listed in Table 6 show a significant change in expression level when using the criteria established by the authors for the less sensitive microarray technique.

There is also one gene in Table 6 whose expression was measured by the more sensitive technique of DD, and its level increased from a qualitative value of 0 to 2, a more than 2-fold increase (spot 2: actin, gamma, cytoplasmic). This increase in mRNA was accompanied by a corresponding increase in protein level, from 3 to 6.

Therefore, although the authors characterize the mRNA and protein levels as having a "poor correlation," this does not reflect a lack of a correlation between a change in mRNA level and a corresponding change in protein level. Only two genes meet the authors' criteria for differentially expressed mRNA level, and of those, one apparently shows a corresponding change in protein level and one does not. Thus, there is little basis for the authors' conclusion relied on by the PTO that "it may be difficult to extrapolate directly from individual mRNA changes to corresponding ones in protein levels [as estimated from 2DE]." (Page 10 of the instant Office Action; emphasis added).

Finally, Applicants submit that Lian *et al.* only teach that protein expression may not correlate with mRNA level in differentiating myeloid cells and does not teach anything regarding such a lack of correlation for genes in general. Myeloid cell differentiation relates to hematopoiesis and is an entirely different biological process from solid tumor development because these two process involve entirely different regulatory mechanisms and molecules. Analysis of surface antigens expressed on myeloid cells of the granulocyte-monocyte-histiocyte series during differentiation in normal and malignant myelomonocytic cells is useful in identifying and classifying human leukemias and lymphomas, but cannot be used in diagnosis of any solid tumors. Therefore, even if the teaching of Lian *et al.* accurately reflects the correlation between mRNA and protein for the particular system studied, it can not apply to the tumor diagnosis assays of the present application.

Fessler *et al.*

The PTO also cites a publication by Fessler *et al.* Fessler is not contrary to Applicants' asserted utility, and actually supports Applicants' assertion that a change in the level of mRNA for a particular protein generally leads to a corresponding change in the level of the encoded protein. As noted above, Applicants make no assertions regarding changes in protein levels when mRNA levels are unchanged, nor does evidence of changes in protein levels when mRNA levels are unchanged have any relevance to Applicants' asserted utility.

Fessler *et al.* studied changes in neutrophil (PMN) gene transcription and protein expression following lipoplysaccharide (LPS) exposure. In Table VIII, Fessler *et al.* list a

comparison of the change in the level of mRNA for 13 up-regulated proteins and 5 down-regulated proteins. Of the 13 up-regulated proteins, a change in mRNA levels is reported for only 3 such proteins. For these 3, mRNA levels are increased in 2 and decreased in the third. Of the 5 down-regulated proteins, a change in mRNA is reported for 3 such proteins. In all 3, mRNA levels also are decreased. Thus, in 5 of the 6 cases for which a change in mRNA levels are reported, the change in the level of mRNA corresponds to the change in the level of the protein. This is consistent with Applicants' assertion that a change in the level of mRNA for a particular protein generally leads to a corresponding change in the level of the encoded protein.

Regarding the remainder of the proteins listed in Table VIII, in 6 instances, protein levels changed while mRNA levels were unchanged. This evidence has no relevance to Applicants' assertion that changes in mRNA levels lead to corresponding changes in protein levels, since Applicants are not asserting that changes in mRNA levels are the only cause of changes in protein levels. In the final 6 instances listed in Table VIII, protein levels changed while mRNA was noted as "absent." This evidence also has no relevance to Applicants' assertion that changes in mRNA levels causes corresponding changes in protein levels. By virtue of being "absent," it is not possible to tell whether mRNA levels were increased, decreased or remained unchanged in PMN upon contact with LPS. Nothing in these results by Fessler *et al.* suggests that a change in the level of mRNA for a particular protein does not generally lead to a corresponding change in the level of the encoded protein. Accordingly, these results are not contrary to Applicants' assertions.

The PTO points to Fessler's statement regarding Table VIII that there was "a poor concordance between mRNA transcript and protein expression changes." (Page 10 of the instant Office Action). As is clear from the above discussion, this statement does not relate to a lack of correlation between a change in mRNA levels leading to a change in protein levels, because in 5 of 6 such instances, changes in mRNA and protein levels correlated well. Instead, this statement relates to observations in which protein levels changed when mRNA was either unchanged or "absent." As such, this statement is an observation that in addition to transcriptional activity, LPS also has post-transcriptional and possibly post-translational activity that affect protein levels,

an observation which is not contrary to Applicants' assertions. Accordingly, Fessler's results are consistent with Applicants' assertion that a change in mRNA level of for a particular protein generally leads to a corresponding change in the level of the encoded protein, since 5 of 6 genes demonstrated such a correlation.

Greenbaum *et al.*

The Examiner asserts that Greenbaum *et al.* "cautions against assuming that mRNA levels are generally correlative of protein levels." (Page 10 of the instant Office Action).

Applicants note that Greenbaum *et al.* compared the expression of a number of different mRNAs and their corresponding proteins in yeast cells. Greenbaum *et al.* did not compare the change of expression of specific mRNAs and their corresponding proteins in cancer cells versus normal cells. Accordingly, this reference is also not relevant to the issue at hand. Nevertheless, Greenbaum states that logically "we would assume that those ORFs that show a large degree of variation in their expression are controlled at the transcriptional level. The variability of the mRNA expression is indicative of the cell controlling the mRNA expression at different points of the cell cycle to achieve the resulting and desired protein. **Thus, we would expect and we found a high degree of correlation (r-0.89) between the reference mRNA and protein levels for these particular ORFs: the cell has already put significant energy into dictating the final level of protein through tightly controlling the mRNA expression**" (page 117.5, col. 1; emphasis added). Furthermore, Greenbaum states that "we found that ORFs that have higher than average levels of ribosomal occupancy – that is that a large percentage of their cellular mRNA concentration is associated with ribosomes (being translated) – have well correlated mRNA and protein expression levels. (Figure 2)." (page 117.5, col. 2; emphasis added). Therefore, contrary to the Examiner's assertion, Greenbaum does find high levels of correlation between mRNA and protein expression in yeast cells. In particular, Greenbaum demonstrates that a high degree of correlation is found for those genes which show a large degree of variability in mRNA expression – that is, for those genes which show changes in mRNA expression, the change in mRNA expression is correlated with a change in protein expression.

The Patent Office has failed to meet its initial burden of proof that Applicant's claims of utility are not substantial or credible. The arguments presented by the Examiner, in combination with the Pennica *et al.*, Konopka *et al.*, Chen *et al.*, Hu *et al.*, LaBaer, Haynes *et al.*, Gygi *et al*, Lian *et al.*, Fessler *et al.* and Greenbaum *et al.* papers, do not provide sufficient reasons to doubt the statements by Applicants that PRO1303 has utility. As discussed in Applicants' Brief filed April 24, 2006, the law does not require the existence of a "necessary" correlation between mRNA and protein levels. According to the authors themselves in the cited references, the data confirm that there is a general trend between protein expression and transcript levels, which meets the "more likely than not standard" and show that a positive correlation exists between mRNA and protein. Therefore, Applicants submit that the Examiner's reasoning is based on a misrepresentation of the scientific data presented in the above cited reference and application of an improper, heightened legal standard. In fact, contrary to what the Examiner contends, the art indicates that, if a gene is overexpressed in cancer, it is more likely than not that the encoded protein will also be expressed at an elevated level.

It is "more likely than not" for increased mRNA levels to predict increased protein levels

Applicants have submitted ample evidence to show that, in general, if a gene is amplified in cancer, it is more likely than not that the encoded protein will be expressed at an elevated level. First, the articles by Orntoft *et al.*, Hyman *et al.*, and Pollack *et al.*, (made of record in Applicants' Response filed July 5, 2005) collectively teach that in general, gene amplification increases mRNA expression. Second, the Declaration of Dr. Paul Polakis, principal investigator of the Tumor Antigen Project of Genentech, Inc., the assignee of the present application, shows that, in general, there is a correlation between mRNA levels and polypeptide levels. Thus, taken together, all of the submitted evidence supports Applicants' position that gene amplification is more likely than not predictive of increased mRNA and polypeptide levels.

With respect to the Polakis Declaration, the Examiner asserts that "the instant specification only discloses gene amplification data for PRO1303" not mRNA data. (Page 15 of

the instant Office Action). Applicants respectfully submit that the Polakis Declartion was submitted as evidence that in general, there is a correlation between mRNA levels and polypeptide levels, the correlation between gene amplification and mRNA expression levels having already been demonstrated by the Orntoft *et al.*, Hyman *et al.*, and Pollack *et al.* references.

The Examiner further asserts that "the data is not included in the declaration so that the Examiner could not independently evaluate them" (Page 15 of the instant Office Action). Applicants emphasize that the opinions expressed in the Polakis Declaration are all based on factual findings. Thus, Dr. Polakis explains that in the course of their research using microarray analysis, he and his co-workers identified approximately 200 gene transcripts that are present in human tumor cells at significantly higher levels than in corresponding normal human cells. Subsequently, antibodies binding to about 30 of these tumor antigens were prepared, and mRNA and protein levels were compared. In approximately 80% of the cases, the researchers found that increases in the level of a particular mRNA correlated with changes in the level of protein expressed from that mRNA when human tumor cells are compared with their corresponding normal cells. Dr. Polakis' statement that "an increased level of mRNA in a tumor cell relative to a normal cell typically correlates to a similar increase in abundance of the encoded protein in the tumor cell relative to the normal cell" is based on factual, experimental findings, clearly set forth in the Declaration. Accordingly, the Declaration is not merely conclusive, and the fact-based conclusions of Dr. Polakis would be considered reasonable and accurate by one skilled in the art.

Furthermore, without acquiescing to the propriety of this rejection, and merely to expedite prosecution in this case, **Applicants present a second Declaration by Dr. Polakis (Polakis II) that presents evidentiary data in Exhibit B**. Exhibit B of the Polakis II Declaration identifies 28 gene transcripts out of 31 gene transcripts (i.e., greater than 90%) that showed good correlation between tumor mRNA and tumor protein levels. As Dr. Polakis' Declaration (Polakis II) says "[a]s such, in the cases where we have been able to quantitatively measure both (i) mRNA and (ii) protein levels in both (i) tumor tissue and (ii) normal tissue, we have observed that in the vast majority of cases, there is a very strong correlation between increases in mRNA

expression and increases in the level of protein encoded by that mRNA." Accordingly, Dr. Polakis has provided the facts to enable the Examiner to draw independent conclusions.

Applicants further enclose a Declaration by Dr. Randy Scott ("the Scott Declaration"). Dr. Scott was a co-founder of Incyte Pharmaceuticals, Inc., the world's first genomic information business, and is currently the Chairman and Chief Executive Officer of Genomic Health, Inc., a life sciences company located in Redwood City, California, which provides individualized information on the likelihood of disease recurrence and response to certain types of therapy using gene expression profiling. Based on his more than 15 years of personal experience with the DNA microarray technique and its various uses in the diagnostic and therapeutic fields, and his familiarity with the relevant art, Dr. Scott unequivocally confirms that, as a general rule, there is a good correlation between mRNA and protein levels in a particular tissue.

As stated in paragraph 10 of the Scott Declaration:

> One reason for the success and wide-spread use of the DNA microarray technique, which has led to the emergence of a new industry, is that generally there is a good correlation between mRNA levels determined by microarray analysis and expression levels of the translated protein. Although there are some exceptions on an individual gene basis, **it has been a consensus in the scientific community that elevated mRNA levels are good predictors of increased abundance of the corresponding translated proteins in a particular tissue**. Therefore, diagnostic markers and drug candidates can be readily and efficiently screened and identified using this technique, without the need to directly measure individual protein expression levels. (emphasis added).

Therefore, if a gene, such as the gene encoding the PRO1303 polypeptide, has been identified to be over-expressed in a certain disease, such as lung or colon cancer, it is more likely than not that the protein product is also overexpressed in the disease.

Both Polakis Declarations (Polakis I and II) and the Scott Declaration are further supported by the teachings in Molecular Biology of the Cell, a leading textbook in the field (Bruce Alberts, *et al.*, Molecular Biology of the Cell (3rd ed. 1994) (copy enclosed, herein after Cell 3rd) and (4th ed. 2002) (copy enclosed, herein after Cell 4th). Figure 9-2 of Cell 3rd shows the steps at which eukaryotic gene expression can be controlled. The first step depicted is

transcriptional control. Cell 3rd provides that "[f]or most genes transcriptional controls are paramount. This makes sense because, of all the possible control points illustrated in Figure 9-2, only transcriptional control ensures that no superfluous intermediates are synthesized." Cell 3rd at 403 (emphasis added). In addition, the text states that "Although controls on the initiation of gene transcription are the predominant form of regulation for most genes, other controls can act later in the pathway from RNA to protein to modulate the amount of gene product that is made." Cell 3rd at 453 (emphasis added). Thus, as established in Cell 3rd, the predominant mechanism for regulating the amount of protein produced is by regulating transcription initiation.

In Cell 4th, Figure 6-3 on page 302 illustrates the basic principle that there is a correlation between increased gene expression and increased protein expression. The accompanying text states that "a cell can change (or regulate) the expression of each of its genes according to the needs of the moment – *most obviously by controlling the production of its mRNA*." Cell 4th at 302 (Emphasis added). Similarly, Figure 6-90 on page 364 of Cell 4th illustrates the path from gene to protein. The accompanying text states that while potentially each step can be regulated by the cell, "the initiation of transcription is the most common point for a cell to regulate the expression of each of its genes." Cell 4th at 364 (Emphasis added). This point is repeated on page 379, where the authors state that of all the possible points for regulating protein expression, "[f]or most genes transcriptional controls are paramount." Cell 4th at 379 (Emphasis added).

Further support for Applicants' position can be found in the textbook, Genes VI, (Benjamin Lewin, Genes VI (1997)) (copy enclosed) which states "having acknowledged that control of gene expression can occur at multiple stages, and that production of RNA cannot inevitably be equated with production of protein, it is clear that the overwhelming majority of regulatory events occur at the initiation of transcription." *Genes VI* at 847-848 (emphasis added).

Additional support is also found in Zhigang *et al.*, World Journal of Surgical Oncology 2:13, 2004 (copy enclosed). Zhigang studied the expression of prostate stem cell antigen (PSCA) protein and mRNA to validate it as a potential molecular target for diagnosis and treatment of human prostate cancer. The data showed "a high degree of correlation between PSCA protein and mRNA expression" *Zhigang* at 4. Of the samples tested, 81 out of 87 showed a high degree

of correlation between mRNA expression and protein expression. The authors conclude that "it is demonstrated that PSCA protein and mRNA overexpressed in human prostate cancer, and that the increased protein level of PSCA resulted from the upregulated transcription of its mRNA." *Zhigang* at 6. Even though the correlation between mRNA expression and protein expression occurred in 93% of the samples tested, not 100%, the authors state that "PSCA may be a promising molecular marker for the clinical prognosis of human Pca and a valuable target for diagnosis and therapy of this tumor." *Id.* at 7.

Further, Meric *et al.*, Molecular Cancer Therapeutics, vol. 1, 971-979 (2002) (copy enclosed) states the following:

> The **fundamental principle** of molecular therapeutics in cancer is to exploit the differences in gene expression between cancer cells and normal cells...[M]ost efforts have concentrated on identifying differences in gene expression at the level of mRNA, which can be attributable to either DNA amplification or to differences in transcription. Meric *et al.* at 971 (emphasis added).

Those of skill in the art would not be focusing on differences in gene expression between cancer cells and normal cells if there were no correlation between gene expression and protein expression.

Together, the declarations of Polakis, the accompanying references, and the excerpts and references provided above all establish that the accepted understanding in the art is that there is a reasonable correlation between changes in gene expression and the level of the encoded protein.

In addition to the supporting references previously submitted by Applicants, Applicants submit the following references to further support the assertion that changes in mRNA levels generally lead to corresponding changes in the level of the encoded polypeptide.

In a study by Wang *et al.* (Urol. Res. 2000; 28(5):308-15) (attached as Exhibit 3) the authors report that down-regulation of E-cadherin protein has been shown in various human tumors. *Id.* at Abstract. In the reported study, the authors examined the expression of cadherins and associated catenins at the mRNA level in paired tumor and non-neoplastic primary prostate cultures. They report that "[s]ix of seven cases of neoplastic cultures showed moderately-to-markedly decreased levels of E-cadherin and P-cadherin mRNA. Similar losses of alpha-catenin

and beta-catenin mRNA were also observed." *Id.* As Applicants' assertion would predict, the authors state that the mRNA measures showed "good correlation" with the results from protein measures. The authors conclude by stating that "this paper presents a coordinated down-regulation in the expression of E-cadherin and associated catenins at the mRNA and protein level in most of the cases studied." *Id.*

In a more recent study by Munaut *et al.* (Int. J. Cancer. 2003; 106(6):848-55) (attached as Exhibit 4) the authors report that vascular endothelial growth factor (VEGF) is expressed in 64-95% of glioblastomas (GBMs), and that VEGF receptors (VEGFR-1, its soluble form sVEGFR-1, VEGFR-2 and neuropilin-1) are expressed predominantly by endothelial cells. *Id.* at Abstract. The authors explain that infiltrating tumor cells and newly-formed capillaries progress through the extracellular matrix by local proteolysis involving matrix metalloproteinases (MMPs). In the present study, the authors "used quantitative RT-PCR, Western blot, gelatin zymography and immunohistochemistry to study the expression of VEGF, VEGFR-1, VEGFR-2, sVEGFR-1, neuropilin-1, MT1-MMP, MMP-2, MMP-9 and TIMP-2 in 20 human GBMs and 5 normal brains. The expression of these MMPs was markedly increased in most GBMs with excellent correlation between mRNA and protein levels." *Id.* Thus, the results support Applicants' assertion that changes in mRNA level lead to corresponding changes in protein level.

In another recent study, Hui *et al.* (Leuk. Lymphoma. 2003; 44(8):1385-94 (abstract attached as Exhibit 5) used real-time quantitative PCR and immunohistochemistry to evaluate cyclin D1 mRNA and protein expression levels in mantle cell lymphoma (MCL). *Id.* at Abstract. The authors report that seven of nine cases of possible MCL showed overexpression of cyclin D1 mRNA, while two cases showed no cyclin D1 mRNA increase. *Id.* Similarly, "[s]ix of the seven cyclin D1 mRNA overexpressing cases showed increased cyclin D1 protein on tissue array immunohistochemistry; one was technically suboptimal." *Id.* The authors conclude that the study "demonstrates good correlation and comparability between measure of cyclin D1 mRNA ... and cyclin D1 protein." *Id.* Thus, this reference supports Applicants' assertion.

In a recent study by Khal *et al.* (Int. J. Biochem. Cell Biol. 2005; 37(10):2196-206) (abstract attached as Exhibit 6) the authors report that atrophy of skeletal muscle is common in

patients with cancer and results in increased morbidity and mortality. *Id.* at Abstract. To further understand the underlying mechanism, the authors studied the expression of the ubiquitin-proteasome pathway in cancer patient muscle using a competitive RT-PCR to measure expression of mRNA for proteasome subunits C2 and C5, while protein expression was determined by western blotting. "Overall, both C2 and C5 gene expression was increased by about three-fold in skeletal muscle of cachectic cancer patients (average weight loss 14.5+/-2.5%), compared with that in patients without weight loss, with or without cancer. ... There was a good correlation between expression of proteasome 20Salpha subunits, detected by western blotting, and C2 and C5 mRNA, showing that increased gene expression resulted in increased protein synthesis." These findings support Applicants' assertion that changes in mRNA level lead to changes in protein level.

Maruyama *et al.* (Am. J. Patho. 1999; 155(3):815-22) (attached as Exhibit 7) investigated the expression of three Id proteins (Id-1, Id-2 and Id-3) in normal pancreas, in pancreatic cancer and in chronic pancreatitis (CP). The authors report that pancreatic cancer cell lines frequently coexpressed all three Ids, "exhibiting good correlation between Id mRNA and protein levels." *Id.* at Abstract. In addition, the authors teach that all three Id mRNA levels were expressed at high levels in pancreatic cancer samples compared to normal or CP samples. At the protein level, Id-1 and Id-2 staining was faint in normal tissue, while Id-3 ranged from weak to strong. In contrast, in the cancer tissues "many of the cancer cells exhibited abundant Id-1, Id-2, and Id-3 immunoreactivity," and Id-1 and Id-2 protein was increased significantly in the cancer cells by comparison to the respective controls, mirroring the overexpression at the mRNA level. Thus, the authors report that in both cell lines and tissue samples, increased mRNA levels leads to an increase in protein overexpression, supporting Applicants' assertion.

Support for Applicants' assertion is also found in an article by Caberlotto *et al.* (Neurosci. Lett. 1999; 256(3):191-4) (abstract attached as Exhibit 8). In a previous study, the authors investigated alterations of neuropeptide Y (NPY) mRNA expression in the Flinders Sensitive Line rats (FSL), an animal model of depression. *Id.* at Abstract. The authors reported that in the current study, that NPY-like immunoreactivity (NPY-LI) was decreased in the hippocampal CA

region, and increased in the arcuate nucleus, and that fluoxetine treatment elevated NPY-LI in the arcuate and anterior cingulate cortex. The authors state that "[t]he results demonstrate a good correlation between NPY peptide and mRNA expression." Thus, increases and decreases in mRNA levels were reflected in corresponding changes in protein level.

Misrachi and Shemesh (Biol. Reprod. 1999; 61(3):776-84) (abstract attached as Exhibit 9) investigated their hypothesis that FSH regulates the bovine cervical prostaglandin E(2) (PGE(2)) synthesis that is known to be associated with cervical relaxation and opening at the time of estrus. *Id.* at Abstract. Cervical tissue from pre-estrous/estrous, luteal, and postovulatory cows were examined for the presence of bovine (b) FSH receptor (R) and its corresponding mRNA. The authors report that bFSHR mRNA in the cervix was maximal during pre-estrus/estrus, and that the level of FSHR protein was significantly higher in pre-estrous/estrous cervix than in other cervical tissues. *Id.* The authors state that "[t]here was a good correlation between the 75-kDa protein expression and its corresponding transcript of 2.55 kb throughout the estrous cycle as described by Northern blot analysis as well as RT-PCR." *Id.* Thus, changes in the level of mRNA for bFSHR led to corresponding changes in FSHR protein levels, a result which supports Applicants' assertion.

In a study by Stein *et al.* (J. Urol. 2000; 164(3 Pt 2):1026-30) (abstract attached as Exhibit 10), the authors studied the role of the regulation of calcium ion homeostasis in smooth muscle contractility. *Id.* at Abstract. The authors investigated the correlation between sarcoplasmic endoplasmic reticulum, calcium, magnesium, adenosine triphosphatase (SERCA) protein and gene expression, and the contractile properties in the same bladder. Partial bladder outlet obstructions were created in adult New Zealand white rabbits, which were divided into control, sham operated and obstructed groups. Stein *et al.* report that "[t]he relative intensities of signals for the Western [protein] and Northern [mRNA] blots demonstrated a strong correlation between protein and gene expression. ... The loss of SERCA protein expression is mediated by down-regulation in gene expression in the same bladder." *Id.* This report supports Applicants' assertion that changes in mRNA level, e.g. a decrease, lead to a corresponding change in the level of the encoded protein, *e.g.*, a decrease.

In an article by Gou and Xie (Zhonghua Jie He He Hu Xi Za Zhi. 2002; 25(6):337-40) (abstract attached as Exhibit 11) the authors investigated the expression of macrophage migration inhibitory factor (MIF) in human acute respiratory distress syndrome(ARDS) by examining the expression of MIF mRNA and protein in lung tissue in ARDS and normal persons. *Id.* at Abstract. The authors report "undetectable or weak MIF mRNA and protein expression in normal lungs. In contrast, there was marked upregulation of MIF mRNA and protein expression in the ARDS lungs." *Id.* This is consistent with Applicants' assertion that a change in mRNA for a particular gene, *e.g.*, an increase, generally leads to a corresponding change in the level of protein expression, *e.g.*, an increase.

These studies are representative of numerous published studies which support Applicants' assertion that changes in mRNA level generally lead to corresponding changes in the level of the expressed protein. Applicants submit herewith an addition 70 references (abstracts attached as Exhibit 12) which support Applicants' assertion.

In addition to these supporting references, Applicants also submit herewith additional references which offer support of Applicants' asserted utility by showing that, in general, mRNA expression levels correlate with protein expression levels.

For example, in an article by Futcher *et al.* (Mol. Cell Biol. 1999; 19(11):7357-68) (attached as Exhibit 13) the authors conducted a study of mRNA and protein expression in yeast. Futcher *et al.* report "a good correlation between protein abundance, mRNA abundance, and codon bias." *Id.* at Abstract.

In a study which is more closely related to Applicants' asserted utility, Godbout *et al.* (J. Biol. Chem. 1998; 273(33)21161-8) (abstract attached as Exhibit 14) studied the DEAD box gene, DDX1, in retinoblastoma and neuroblastoma tumor cell lines. The authors report that "there is a good correlation with DDX1 gene copy number, DDX1 transcript levels, and DDX1 protein levels in all cell lines studied." *Id.* Thus, in these cancer cell lines, DDX1 mRNA and protein levels are correlated.

Similarly, in an article by Papotti *et al.* (Virchows Arch. 2002; 440(5):461-75) (abstract attached as Exhibit 15) the authors examined the expression of three somatostatin receptors

(SSTR) at the mRNA and protein level in forty-six tumors. *Id.* at Abstract. The authors report a "good correlation between RT-PCR [mRNA level] and IHC [protein level] data on SSTR types 2, 3, and 5." *Id.*

Van der Wilt *et al.* (Eur. J. Cancer. 2003; 39(5):691-7) (abstract attached as Exhibit 16) studied deoxycytidine kinase (dCK) in seven cell lines, sixteen acute myeloid leukemia samples, ten human liver samples, and eleven human liver metastases of colorectal cancer origin. *Id.* at Abstract. The authors report that "enzyme activity and protein expression levels of dCK in cell lines were closely related to the mRNA expression levels" and that there was a "good correlation between the different dCK measurements in malignant cells and tumors." *Id.*

Grenback *et al.* (Regul. Pept. 2004; 117(2):127-39) (abstract attached as Exhibit 17) studied the level of galanin in human pituitary adenomas using a specific radioimmunoassay. *Id.* at Abstract. The authors report that "[i]n the tumors analyzed with in situ hybridization there was a good correlation between galanin peptide levels and galanin mRNA expression." *Id.*

Similarly, Shen *et al.* (Blood. 2004; 104(9):2936-9) (abstract attached as Exhibit 18) examined the level of B-cell lymphoma 2 (BCL2) protein expression in germinal center (GC) B-cells and diffuse large B-cell lymphoma (DLBCL). *Id.* at Abstract. The authors report that "GC cells had low expression commensurate with the low protein expression level" and that in DLBCL the level of BCL2 mRNA and protein expression showed "in general, a good correlation." *Id.*

Likewise, in an article by Fu *et al.* (Blood 2005; 106(13):4315-21) (abstract attached as Exhibit 19) the authors report that six mantle cell lymphomas studied "expressed either cyclin D2 (2 cases) or cyclin D3 (4 cases)." *Id.* at Abstract. "There was a good correlation between cyclin D protein expression and the corresponding mRNA expression levels by gene expression analysis." *Id.*

These examples are only a few of the many references Applicants could cite in rebuttal to the PTO's arguments. Applicants submit herewith 26 additional references (abstracts attached as Exhibit 20) which also support Applicants' assertion in that they report a correlation between the

level of mRNA and corresponding protein, contrary to the assertion of the PTO that mRNA and protein levels are not correlated.

Applicants note that the new references submitted in the Information Disclosure Statement focus on the correlation between mRNA expression and protein expression levels, and for the most part do not examine gene amplification. However, those few references that actually looked at gene amplification **did** find a correlation between gene amplification and increased mRNA and protein expression levels.

For example, Bea *et al.* (Cancer Res. 2001; 61(6):2409-12) (abstract attached in Exhibit 12) investigated gene amplification, mRNA expression, and protein expression of the putative oncogene BMI-1 in human lymphoma samples. The authors found BMI-1 gene amplification in four mantle cell lymphomas (MCLs). Bea *et al.* report that "[t]he **four tumors with gene amplification showed significantly higher mRNA levels** than other MCLs and NHLs with the BMI-1 gene in germline configuration" (Abstract; emphasis added). Applicants note that the fact that five additional MCLs also showed very high mRNA levels without gene amplification does not disprove Applicants' position, because one of skill in the art would understand that there can be more than one cause of mRNA overexpression. The issue is not whether mRNA overexpression is always, or even typically caused by gene amplification, but rather, whether gene amplification typically leads to overexpression. Bea *et al.* further note that the four MCLS with gene amplification of *BMI-1* "showed significantly higher levels of mRNA **and protein expression** compared with other lymphomas with *BMI-1* in germline configuration" (page 2411, col. 1; emphasis added). Thus Bea *et al.* supports Applicants' assertion that gene amplification is correlated with both increased mRNA and protein expression.

Godbout *et al.* (J. Biol. Chem. 1998; 273(33)21161-8) (abstract attached as Exhibit 14) studied the DEAD box gene, DDX1, in retinoblastoma and neuroblastoma tumor cell lines. The authors report that "**there is a good correlation with DDX1 gene copy number, DDX1 transcript levels, and DDX1 protein levels in all cell lines studied**" (Abstract). Thus Godbout

et al. also supports Applicants' assertion that gene amplification is correlated with both increased mRNA and protein expression.

Applicants note that while Fu *et al.* (Blood 2005; 106(13):4315-21) (abstract attached as Exhibit 19) found increased mRNA and protein expression of cyclin D2 and cyclin D3 in the absence of gene amplification, this result proves only that increased mRNA and protein expression levels can result from causes other than gene amplification. As Applicants do not assert that gene amplification is the only cause of increased mRNA and protein expression levels, this result does not disprove Applicants assertion that that increased gene amplification, in general, is correlated with increased mRNA and protein expression.

In summary, Applicants submit herewith a total of 118 references in addition to the declarations and references already of record which support Applicants' asserted utility, either directly or indirectly. These references support the assertion that in general, a change in mRNA expression level for a particular gene leads to a corresponding change in the level of expression of the encoded protein. As Applicants have previously acknowledged, the correlation between changes in mRNA level and protein level is not exact, and there are exceptions (*see, e.g.*, abstracts attached as Exhibit 21). However, Applicants remind the PTO that the asserted utility does not have to be established to a statistical certainty, or beyond a reasonable doubt. *See M.P.E.P.* at § 2107.02, part VII (2004). Therefore, the fact that there are exceptions to the correlation between changes in mRNA and changes in protein does not provide a proper basis for rejecting Applicants' asserted utility. Applicants submit that considering the evidence as a whole, with the overwhelming majority of the evidence supporting Applicants' asserted utility, a person of skill in the art would conclude that Applicants' asserted utility is "more likely than not true."

In view of the above, Applicants request the Examiner to reconsider and withdraw the rejection of Claims 28-32 under 35 U.S.C. § 101 and 35 U.S.C. § 112, first paragraph.

II. Claim Rejections Under 35 U.S.C. §102(e)

Claims 28-32 remain rejected under 35 U.S.C. §102(e) as allegedly being anticipated by Ni *et al.* (U.S. Patent No. 6,566,498). Ni *et al.* teach an isolated human secreted polypeptide consisting of SEQ ID NO:6, which has two regions of 100% identity to SEQ ID NO:194, one region of 62 amino acids at the N-terminus, and one of 93 amino acids at the C-terminus of SEQ ID NO:194. The Examiner asserts that "[t]he proteins disclosed by Ni *et al.* have significant lengths of identical amino acid sequences, and therefore antibodies that specifically bind to the polypeptide disclosed by Ni et al. would specifically bind to the PRO1303 of the instant invention." (Page 19 of the instant Office Action).

Applicants respectfully point out that, as discussed in the Response filed July 5, 2005, and in the Appeal Brief filed May 2, 2006, Claim 28, and consequently, those claims dependent from Claim 28, recites "an antibody that specifically binds to the polypeptide of SEQ ID NO:194." (Emphasis added). Therefore, Claim 28 and the claims dependent from Claim 28, carrying its recitations, clearly refer to an antibody that is able to bind to a specific epitope of the PRO1303 polypeptide *without* cross reacting with another epitope, including those found in the sequence disclosed in Ni *et al.* In view of this, the Examiner errs in assuming that the antibodies claimed in the present application would display significant binding to the polypeptide of Ni *et al.*, and thus overlap with the antibodies of Ni *et al.* As a result of the requirement of specific binding, the claims pending in this application do not encompass antibodies that specifically bind to epitopes found in the polypeptide of Ni *et al.*

Clearly there exist specific epitopes in the SEQ ID NO:194 protein that are not found in the Ni protein. Applicants note that, as shown in the sequence alignment provided by the Examiner in the previous Office Action mailed April 5, 2005, SEQ ID NO:194 contains a 93 amino acid region in the middle of the protein that is not present in SEQ ID NO:6 of Ni *et al.* Thus this 93 amino acid region of SEQ ID NO:194 contains numerous epitopes not found in the protein of Ni *et al.* Accordingly, one of ordinary skill in the art would readily understand what is meant by antibodies which specifically bind to SEQ ID NO:194 (and not, for example, to the

polypeptide of Ni *et al.*). Such antibodies would clearly include, for example, antibodies that bind to epitopes found within the central 93 amino acid region of SEQ ID NO:194. One of skill in the art would understand that specific epitopes of the PRO1303 polypeptide may also include residues from the overlapping regions, as part of a non-linear epitope.

One of ordinary skill in the art would further understand how to make and use such antibodies. The specification provides methods to determine whether an antibody specifically binds to epitopes possessed by SEQ ID NO:194. Routine methods of determining antibody binding specificities, including immunoprecipitation, or competitive binding assays such as radioimmunoassay (RIA) or enzyme-linked immunoabsorbent assay (ELISA), are disclosed in the specification at, for example, page 373, lines 32-35. Methods of determining the binding affinities of antibodies using Scatchard analysis are disclosed at page 373, lines 35-36. In addition, a method of using competitive binding assays to determine if a peptide shares an antigenic determinant for a particular antibody with a PRO polypeptide is disclosed in the specification at page 488, lines 25-29.

The Examiner asserts that "the art accepted meaning of an antibody that "specifically" binds is that the binding is relatively strong, and specific in that said antibody 'recognizes' a particular protein sequence and three-dimensional structure. There is no exclusion in the art accepted meaning which would define such binding as never binding to another protein having an identical epitope." (Page 19 of the instant Office Action).

Applicants respectfully point out that the claims do not recite antibodies which specifically bind to an epitope. Rather, the claims recite antibodies that specifically bind to SEQ ID NO:194. That is, the recited antibodies recognize not merely specific epitopes, but epitopes which are specific to SEQ ID NO:194 and not, for example, to the protein of Ni *et al.* Thus the issue to consider is not whether the antibodies of Ni *et al.* bind to specific epitopes (as most antibodies do), but whether they bind to specific epitopes of SEQ ID NO:194, as would be demonstrated, for example, by a failure of the Ni *et al.* protein to significantly compete with SEQ ID NO:194 for binding to the Ni *et al.* antibody.

The reference antibodies of Ni *et al.* do not anticipate the claims because these antibodies were raised to the Ni *et al.* protein and therefore would not be expected to have stronger binding to SEQ ID NO:194 than the Ni *et al.* protein. Applicants submit that indeed there may be antibodies that are capable of binding to epitopes present in both the PRO1303 polypeptide and the Ni *et al.* protein. However, the present invention does not claim such antibodies. Applicants respectfully point out that Claim 28 recites antibodies that specifically bind to SEQ ID NO:1303. As discussed above, the term "specifically binds" in this context refers to antibody that is able to bind to a specific epitope of the PRO1303 polypeptide of SEQ ID NO:194 *without* cross reacting with other epitopes, including those found in the sequence disclosed in Ni *et al.* One of ordinary skill in the art, in making antibodies that specifically bind to SEQ ID NO:194, would therefore look for epitopes that are specific to SEQ ID NO:194, and would have as a logical consequence been led to the unique 93 amino acid region of SEQ ID NO:194. It is understood in the art that a 93 amino acid region is large enough to comprise an independent folding domain, which may well have an additional function not present in the shorter variant. Because it is well known in the art that splice variants may have different functions and expression patterns, the skilled artisan would want to be able to distinguish between such variants. The skilled artisan would further understand that it would be simple to make antibodies that would specifically bind only the longer variant, by making antibodies to the unique splice region of the longer protein. Thus there is nothing unreasonable or untenable in the idea of generating antibodies that 'specifically bind' to proteins having entire domains not found in a related variant. The skilled artisan would further understand that antibodies could be tested for specific binding by, for example, doing a Western blot of a cell lysate and confirming that there were no strong bands other than the one corresponding to the protein of SEQ ID NO:194.

Accordingly, Applicants respectfully submit that Ni *et al.* is not prior art under 102(e) and request the Examiner to reconsider and withdraw the present rejection.

CONCLUSION

All claims pending in the present application are believed to be in *prima facie* condition for allowance, and an early action to that effect is respectfully solicited.

Please charge any additional fees, including any fees for additional extension of time, or credit overpayment to Deposit Account No. **08-1641** (Attorney's Docket No. **39780-2830 P1C15**). Please direct any calls in connection with this application to the undersigned at the number provided below.

Respectfully submitted,

Date: November 28, 2005

By: _____
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